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SHORT COMMUNICATION

Ebola Virus Localization in the Macaque Reproductive Tract during Acute Ebola Virus Disease

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Sexual transmission of Ebola virus (EBOV) has been demonstrated more than a year after recovery from the acute phase of Ebola virus disease (EVD). The mechanisms underlying EBOV persistence and sexual transmission are not currently understood. Using the acute macaque model of EVD, we hypothesized EBOV would infect the reproductive tissues and sought to localize the infection in these tissues using immunohistochemistry and transmission electron microscopy. In four female and eight male macaques that succumbed to EVD between 6 and 9 days after EBOV challenge, we demonstrate widespread EBOV infection of the interstitial tissues and endothelium in the ovary, uterus, testis, seminal vesicle, epididymis, and prostate gland, with minimal associated tissue immune response or organ pathology. Given the widespread involvement of EBOV in the reproductive tracts of both male and female macaques, it is reasonable to surmise that our understanding of the mechanisms underlying sexual transmission of EVD and persistence of EBOV in immune-privileged sites would be facilitated by the development of a nonhuman primate model in which the macaques survived past the acute stage into convalescence. *(Am J Pathol 2018, 188: 550–558; https://doi.org/10.1016/j.ajpath.2017.11.004)*

Sexual transmission of filoviruses has been suspected for as long as these viruses have been recognized as human pathogens. During the first recorded outbreak of Marburg virus disease in 1967, sexual transmission from an infected man to a woman was presumed but never proved.[1] Ebola virus (EBOV) was detected in the semen of a researcher infected while handling diagnostic samples from the first recorded EBOV outbreak in 1976.[2] The 2014 to 2016 EBOV outbreak in West Africa again raised the question of sexual transmission of filoviruses and the potential for asymptomatic infections and EBOV persistence in immune-privileged sites.[3–6] Findings from >17,000 survivors of the outbreak in West Africa suggest sexual transmission is rare. To date, only two recorded incidences of sexual transmission of EBOV from that outbreak have been documented.[7,8] However, the implications of EBOV transmission by this route are significant given an Ebola virus disease (EVD) mortality rate of >40%.[9,10] We hypothesized that EBOV would infect the reproductive tissues in the macaque model of acute EVD. The purpose of this study was to identify and localize the virus within the reproductive tissues in the macaque model, typically acutely fatal between 6 and 10 days after i.m. challenge. Using this EVD model, we demonstrate EBOV infection of the

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Table 1 Signalment, EBOV in Peripheral Blood, and EBOV Distribution in the Reproductive Tract of Female and Male Macaques

Animal ID	Macaque species	Age, months	Necropsy after inoculation, days	EBOV vRNA, copy/mL (log10)	EBOV IHC results						TEM results
					Ovary	Uterus	Testis	Prostate gland	Seminal vesicle	Epididymis	
1	Cynomolgus	46	7	10.6	Stromal/vascular	Stromal/vascular					Stromal/EC
2	Cynomolgus	53	8	9.96	Stromal/vascular	Stromal/vascular					Stromal
3	Cynomolgus	53	7	10.1	Stromal/vascular	Stromal/vascular					Stromal/EC
4	Rhesus	35	7	10.4	Stromal/vascular	Stromal/vascular					Stromal
5	Cynomolgus	48	6	10.3			Stromal/vascular	Stromal/vascular	Stromal/vascular	Stromal/vascular	Stromal/EC
6	Cynomolgus	44	8	10			Stromal/vascular	Stromal/vascular	Stromal/vascular	Stromal/vascular	Stromal/EC
7	Rhesus	44	9	8.83			Stromal/vascular	Stromal/vascular	Stromal/vascular	NP	Stromal/EC
8	Rhesus	48	7	9.78			Stromal/vascular	Stromal/vascular	Stromal/vascular	NP	Stromal/EC
9	Rhesus	58	8	9.95			Stromal/vascular	Stromal/vascular	Stromal/vascular	Stromal/vascular	Stromal/EC
10	Rhesus	56	7	10.2			Stromal/vascular	Stromal/vascular	Stromal/vascular	Stromal/vascular	Stromal/EC
11	Rhesus	35	7	10.1			Stromal/vascular	Stromal/vascular	Stromal/vascular	Stromal/vascular	Stromal/EC
12	Rhesus	38	6	10.6			Stromal/vascular	Stromal/vascular	Stromal/vascular	Stromal/vascular	NP

EBOV, Ebola virus; EC, endothelial cell; ID, identification; IHC, immunohistochemistry; NP, not performed; TEM, transmission electron microscopy; vRNA, viral RNA.

interstitial or supporting connective tissues of the male and female reproductive organs, with minimal associated tissue immune response or organ pathology.

Twelve macaques, seven rhesus (*Macaca mulatta*; one female and six males) and five cynomolgus (*Macaca fascicularis*; three females and two males), ranging in age from 35 to 58 months, were challenged with 1000 plaque-forming units i.m. of EBOV Makona C05.[11] Macaques were euthanized after meeting end point criteria between 6 and 9 days after EBOV challenge. Each macaque underwent a complete postmortem examination when predetermined end point criteria[12] for EVD were reached. Reproductive tissues were collected from all macaques for routine histology, immunohistochemistry (IHC) for EBOV viral protein 40 (VP40) matrix protein and/or glycoprotein (GP), and transmission electron microscopy examination.[13] Immunohistochemistry for EBOV VP40 and/or GP was positive in the reproductive organs of all 12 macaques. Transmission electron microscopy of the reproductive tissues was performed in 11 of 12 macaques that succumbed to EVD, and widespread EBOV infection of the stromal connective tissues, including endothelial cells, was observed in all macaques examined. A summation of findings can be found in Table 1.

Materials and Methods

Animal Ethics Statement

This study was performed in strict adherence to the *Guide for the Care and Use of Laboratory Animals*[14] of the NIH, the Office of Animal Welfare, and the US Department of Agriculture. All work was approved by the US National Institute of Allergy and Infectious Diseases, Division of Clinical Research Animal Care and Use Committee, and performed at the US National Institute of Allergy and Infectious Diseases Research Facilities. Procedures were performed after animals had been anesthetized by trained personnel under the supervision of veterinary staff. Food and water were available ad libitum.

Animals

This study includes 12 macaques that served as controls in three separate studies that were designed to evaluate the efficacy of a therapeutic agent intended to protect against the development of EVD. The first two experiments are described by Johnson et al.[15] The seven rhesus macaques (*M. mulatta*) and five cynomolgus macaques (*M. fascicularis*) ranged in age from 35 to 58 months, with a

total of four females (three cynomolgus and one rhesus) and eight males (two cynomolgus and six rhesus). These 12 macaques were randomized to control groups before EBOV i.m. challenge with EBOV (Zaire species) in the lateral head of the right triceps muscle using the Makona C05 EBOV isolate at a target dose of 1000 plaque-forming units.[11] All macaques succumbed to EVD between 6 and 9 days after EBOV challenge.

Blood Collection

Nine pre-EBOV challenge blood draws were performed in each macaque, the last at 15 days before EBOV challenge (0 day). After EBOV challenge, blood draws were performed every other day starting at 2 days until 8 days after EBOV challenge. The last blood draw was performed immediately before necropsy. Complete blood cell counts, select serum chemistries, and real-time quantitative PCR for viremia were performed on these samples.

Viremia by Quantitative RT-PCR

Whole blood was inactivated in TRIzol LS (Thermo Fisher Scientific, Waltham, MA) in accordance with established methods for inactivation of risk group 4 pathogens. Briefly, total RNA was isolated using the QIAamp Viral RNA Mini Kit (Qiagen, Germantown, MD) using 700 μL of TRIzol LS inactivated sample that was added to 280 μL of Qiagen Buffer AVL containing carrier RNA. The sample was eluted in 70 μL of Buffer AVE, aliquoted, and frozen until assayed. Whole blood viral load was measured using BEI Resources Critical Reagents Program (Manassas, VA) EZ1 quantitative RT-PCR kit assay, in accordance with manufacturer's instructions, and reported as viral RNA copies ($\log_{10}$) per mL of sample.

Tissue Processing and Immunohistochemistry

Reproductive tissue collected from females (uterus and ovaries) and males (testes, prostate, seminal vesicles, head, and tail of the epididymides) at necropsy was fixed in 10% neutral-buffered formalin in preparation for routine histopathology and immunohistochemistry. Ebola virus immunohistochemistry was performed on routinely processed tissue with an anti-EBOV VP40 (catalog number 0201-016; IBT Bioservices, Rockville, MD) or anti-EBOV GP antibody (catalog number 0301-015; IBT Bioservices), followed by a biotinylated anti-mouse (catalog number 115-065-166; Jackson Immunoresearch Laboratories, Westgrove, PA; for VP40) or anti-rabbit (catalog number 111-065-144; Jackson Immunoresearch Laboratories; for GP) secondary antibody and an avidin-biotin peroxidase tertiary antibody (catalog number PK-6100; Vector Laboratories, Burlingame, CA). Ebola virus was visualized with 3,3′-diaminobenzidine chromogen (catalog number BDB2004L; Biocare, Pacheco, CA), counterstained with hematoxylin, and assessed by a single pathologist (D.L.P.). Immunohistochemical controls included Ebola virus–infected liver from an EBOV-infected macaque and liver from an EBOV-naïve macaque, as positive and negative controls, respectively. In addition, assay controls (omission of the primary antibody) and isotype controls were performed for EBOV-VP40 (mouse IgG1; catalog number ab18443; Abcam, Cambridge, MA) and EBOV-GP (rabbit IgG; catalog number ab27478; Abcam). The distribution of EBOV IHC staining in the reproductive tissues was similar with anti-EBOV VP40 and anti-EBOV GP.

Electron Microscopy

For conventional thin-section microscopic evaluation, tissues were fixed for 72 hours in 2.5% glutaraldehyde and 2.0% paraformaldehyde (E.M. Sciences, Warrington, PA) in Millonig's sodium phosphate buffer (Tousimis Research, Rockville, MD). Fixed tissues were washed repeatedly in Millonig's buffer and then incubated for 2 hours in 1.0% osmium tetroxide. After rinsing steps in ultrapure water and *en bloc* staining with 2.0% uranyl acetate, tissues were dehydrated in a series of graded ethanols and infiltrated and embedded in Spurr's plastic resin (E.M. Sciences). Embedded blocks were divided into sections using a Leica UC7 ultramicrotome (Leica, Wetzlar, Germany). Sections (70 to 80 nm thick) were collected on 200 mesh copper grids and poststained with Reynold's lead citrate. Samples were examined in an FEI Tecnai Spirit Twin transmission electron microscope (FEI Company, Hillsboro, OR), operating at 80 kV.

Results

Ebola Virus Infects Mesenchymal Cells in the Ovary

Histopathological lesions in the ovary secondary to EVD were not observed in the macaques examined. Inclusion bodies could be seen in the mesenchymal thecal cells surrounding secondary and tertiary ovarian follicles. Immunohistochemical staining for EBOV GP in the ovary was multifocally positive in the thecal cells surrounding secondary and tertiary ovarian follicles and in the vascular endothelium in macaques that succumbed to EVD (Figure 1). Immunohistochemical positivity in the granulosa cells was rare. Ultrastructural examination of the ovary confirmed viral infection of ovarian stromal cells and endothelial cells (Figure 1). Fimbrial epithelial cells of the salpinx (Fallopian tube or oviduct) stained positive multifocally for EBOV VP40 and GP (data not shown).

Ebola Virus Infects the Endometrial Stromal Cells and Smooth Muscle Cells of the Myometrium

Histopathological lesions in the uterus secondary to EVD were not observed in the macaques examined. Immunohistochemical staining for EBOV VP40 revealed widespread positivity in the vasculature, endometrial stromal cells, and smooth muscle cells of the myometrium in macaques that succumbed to EVD (Figure 1). Ultrastructural examination

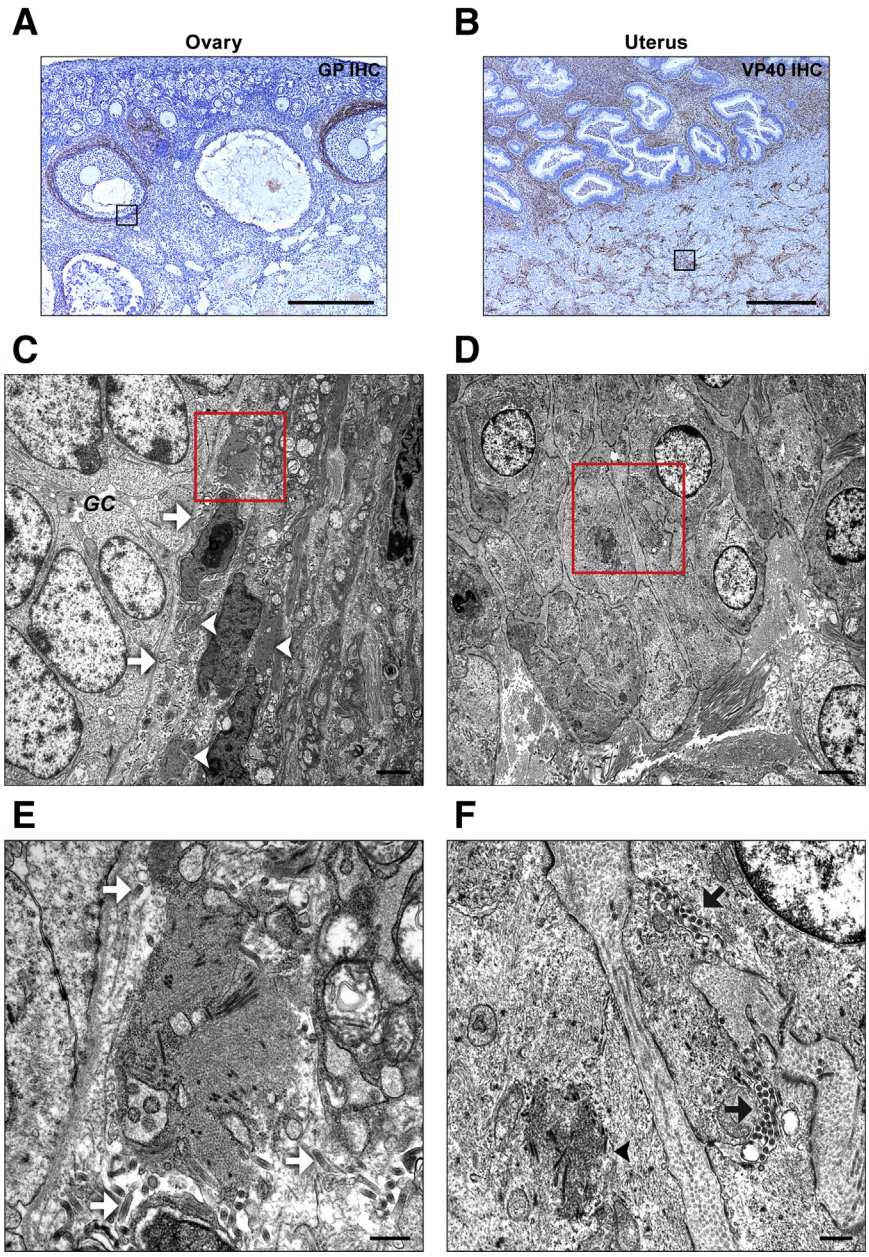

Figure 1 Ovary (**A**, **C**, and **E**) and uterus (**B**, **D**, and **F**). **A:** Section of ovary from a macaque 7 days after Ebola virus (EBOV) challenge immunohistochemically (IHC) stained for EBOV glycoprotein (GP) shows positive cytoplasmic staining (brown) of the stroma, most prominent around secondary and tertiary ovarian follicles, consistent with the theca interna layer. **B:** Section of uterus IHC stained for EBOV viral protein 40 (VP40) matrix protein shows extensive positive cytoplasmic staining (brown) within the endometrial stroma, vasculature, and smooth muscle of the myometrium. **C** and **E:** The **black boxed area** from **A** shows the anatomical orientation of the transmission electron micrographs (TEMs). **C:** An intracytoplasmic EBOV inclusion body (**red boxed area**) forming nucleocapsid structures is present in a mesenchymal cell in the ovary adjacent to a granulosa cell layer (GC; left). Extracellular, filamentous, mature EBOV particles are in the interstitium (**white arrows**). The **white arrowheads** point to additional intracytoplasmic EBOV inclusion bodies in ovarian mesenchymal cells. **E:** Higher magnification of the inclusion body in the **red boxed area** in **C**. An intracytoplasmic inclusion body forming EBOV nucleocapsid structures. **White arrows** point to mature EBOV particles free within the interstitial space. **D** and **F:** The **black boxed area** in **B** represents the anatomical orientation of smooth muscle cells seen in TEMs. **D:** An intracytoplasmic EBOV inclusion body (**red boxed area**) is present in a smooth muscle cell of the myometrium. Adjacent to the smooth muscle cells are mature filamentous EBOV particles free within the interstitial space. **F:** Higher magnification of the **red boxed area** in **D**. Intracytoplasmic EBOV inclusion body (**black arrowhead**) forming nucleocapsid structures within a smooth muscle cell in the myometrium. Adjacent to the smooth muscle cells are filamentous mature EBOV particles free within interstitial space (**black arrows**). Scale bars: 500 μm (**A** and **B**); 2 μm (**C** and **D**); 500 nm (**E** and **F**).

of the uterus confirmed viral infection of endometrial stromal cells and smooth muscles cells of the myometrium (Figure 1).

Ebola Virus Infects Interstitial Mesenchymal Cells in the Testis

Histopathological lesions in the testis were uncommon but included the presence of spermatid giant cells and oligospermia; however, macaque species are seasonal breeders and sperm density must be interpreted in the context of age, social rank, recent changes in social housing,[16] and photoperiod or season.[17] Uncommonly, thrombosis with infarction of testicular stroma occurred secondary to EVD-induced coagulopathy, resulting in areas of ischemic necrosis of the testicular tissue. Immunohistochemistry for EBOV VP40 and GP in the testis revealed widespread positivity in the interstitial connective tissues, including endothelial cells (Figure 2). Ultrastructural examination of the testis confirmed viral infection of interstitial connective tissues, including endothelial cells (Figure 2).

Ebola Virus Infects Interstitial Mesenchymal Cells in the Accessory Male Sex Glands (Prostate Gland, Seminal Vesicle, and Epididymis)

In the prostate gland and seminal vesicle, histopathological lesions secondary to EVD were not observed, despite widespread immunohistochemical positivity for EBOV VP40 and

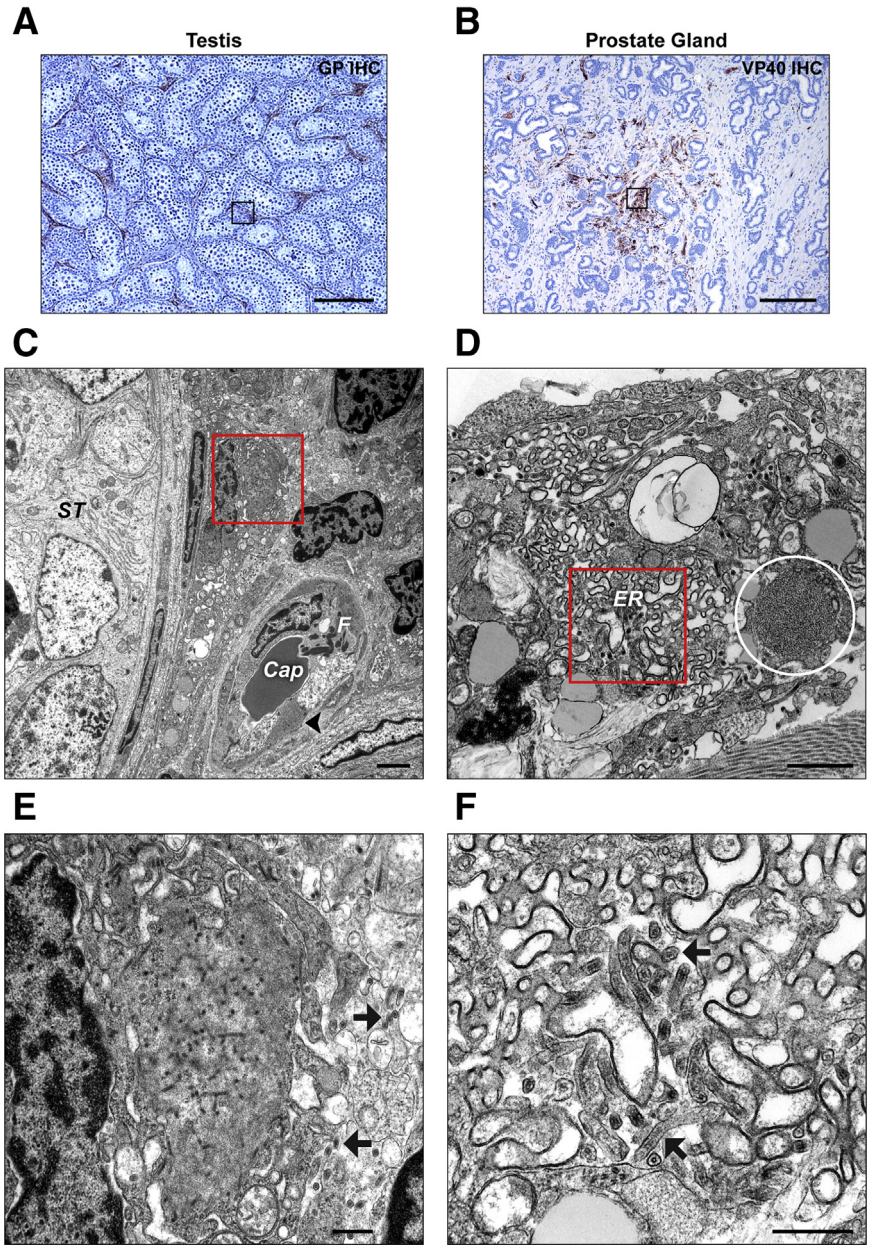

Figure 2 Testis (**A**, **C**, and **E**) and prostate gland (**B**, **D**, and **F**). **A:** Immunohistochemistry (IHC) for Ebola virus (EBOV) glycoprotein (GP) shows positive cytoplasmic staining (brown) within the interstitium of the testis. The spermatogonia and Sertoli cells of the seminiferous tubules are negative for EBOV GP. **B:** Section of prostate gland IHC stained for EBOV viral protein 40 (VP40) matrix protein shows multifocal positive cytoplasmic staining (brown) of the interstitial mesenchymal cells. **C** and **E:** The **black boxed area** in **A** represents the anatomical orientation of the transmission electron micrographic (TEM) images. **C:** Intracytoplasmic EBOV inclusion bodies can be seen within mesenchymal cells (**red boxed area**) and endothelial cells (**black arrowhead**) within the testicular interstitium adjacent to an intact seminiferous tubule (ST; left) in a macaque 6 days after EBOV challenge. The endothelial cells of the capillary (Cap) are multifocally electron lucent (edema). The capillary lumen contains fibrin (F). **E:** Higher magnification of the **red boxed area** in **C**. EBOV nucleocapsid formation can be seen within an intracytoplasmic inclusion body (center) present within an interstitial mesenchymal cell of the testis seen in **C**. Adjacent to this inclusion body are mature EBOV particles free within the interstitial space (**black arrows**). **D** and **F:** The **black boxed area** in **B** represents the anatomical orientation of the image seen in TEMs. **D:** Filamentous EBOV particles are present within a disrupted and expanded smooth endoplasmic reticulum (ER) of an interstitial mesenchymal cell. An EBOV intracytoplasmic inclusion body is present adjacent to the expanded ER (white circle). **F:** Higher magnification of the **red boxed area** in **D**, demonstrating filamentous EBOV particles (**black arrows**) among expanded ER membranes. Scale bars: 200 μm (**A** and **B**); 2 μm (**C**); 1 μm (**D**); 500 nm (**E** and **F**).

GP in the stromal connective tissues (Figures 2 and 3). Epithelial cell positivity for EBOV by IHC in these organs was rare. In the head and tail of the epididymis, histopathological lesions secondary to EVD were nonspecific, but included interstitial edema and, rarely, vascular thrombosis. Similar widespread multifocal positivity for EBOV VP40 and GP was detected in the interstitial connective tissue supporting the tubular epithelium of the epididymis in acutely EVD-affected macaques (Figure 3). Ultrastructural examination of the prostate gland, epididymis, and seminal vesicle confirmed viral infection of interstitial mesenchymal cells, including endothelial cells (Figures 2 and 3). Mature EBOV particles were seen within the disrupted membranes of an expanded endoplasmic reticulum; however, the virus was only observed to bud from the cell surface or plasma membrane. Ebola virus GP is known to accumulate in the endoplasmic reticulum of infected cells,[18,19] which may partially explain the marked expansion of endoplasmic reticulum membranes observed occasionally in EBOV-infected cells. Within the ductus deferens, epithelial positivity for EBOV VP40 protein was observed (data not shown).

Discussion

Using the macaque model of acute EVD, we have demonstrated early and widespread infection of the male and female reproductive tract after i.m. EBOV challenge. Ebola

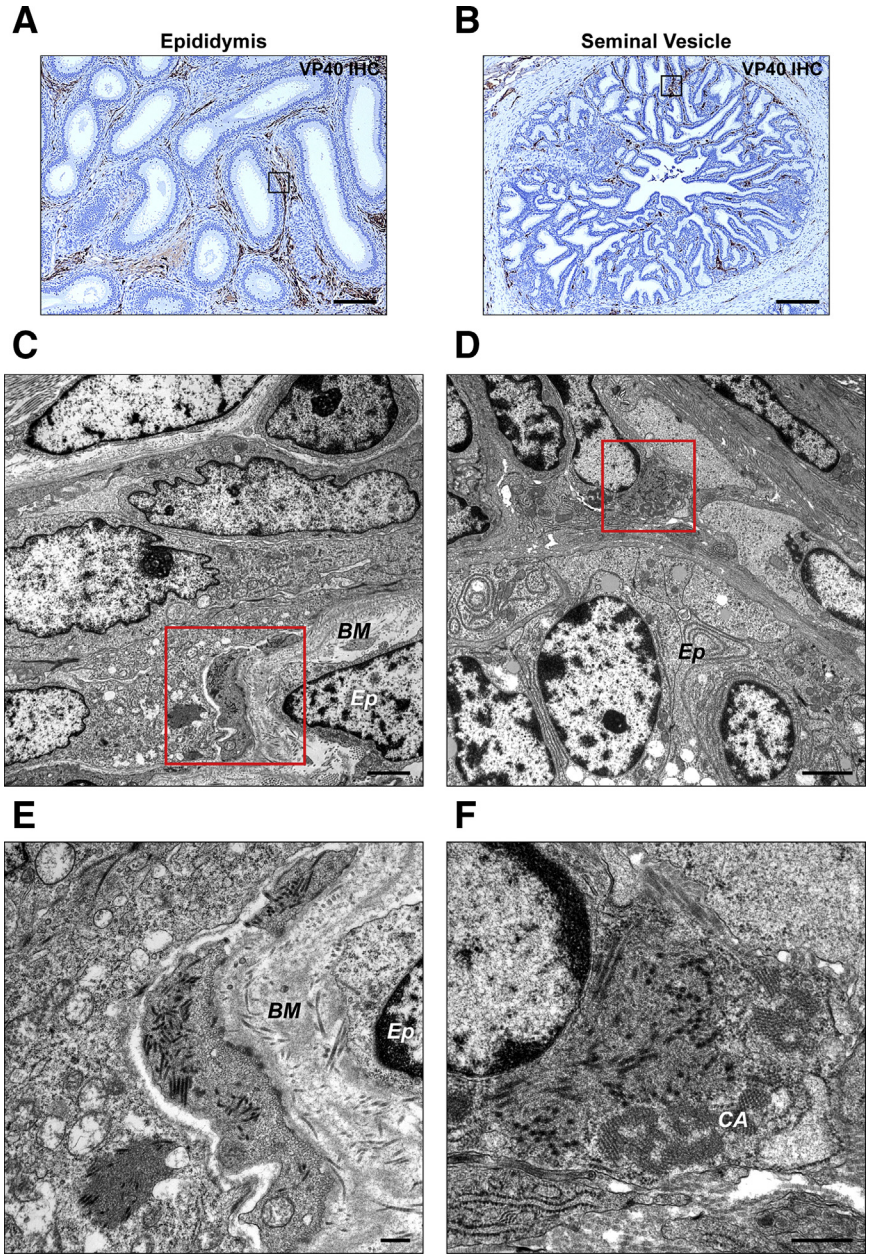

Figure 3 Epididymis (**A**, **C**, and **E**) and seminal vesicle (**B**, **D**, and **F**). **A:** Section of epididymis immunohistochemically (IHC) stained for Ebola virus (EBOV) viral protein 40 (VP40) matrix protein, shows multifocal positive cytoplasmic staining (brown) of the interstitial mesenchymal cells of the epididymis. **B:** Section of seminal vesicle IHC stained for EBOV VP40 matrix protein shows multifocal cytoplasmic positivity (brown) in the mesenchymal cells of the seminal vesicle. **C** and **E:** The **black boxed area** in **A** reveals the anatomical orientation seen in transmission electron micrographs (TEMs). **C:** Four intracytoplasmic EBOV inclusion bodies are present within mesenchymal cells adjacent to the basement membrane (BM) of the epididymal epithelium (Ep). **E:** Higher magnification of the **red boxed area** in **C**. Filamentous EBOV nucleocapsid proteins form an intracytoplasmic inclusion body in an interstitial mesenchymal cell adjacent to the BM of the Ep. **D** and **F:** The **black boxed area** in **B** represents the anatomical orientation of the TEMs. **D:** An intracytoplasmic EBOV inclusion body is present in a mesenchymal cell adjacent to the Ep of the seminal vesicle. **F:** Higher magnification of the **red boxed area** in **D**. An intracytoplasmic EBOV inclusion body demonstrates EBOV nucleocapsid formation in longitudinal and cross section [in cross section appearing as crystalline arrays (CAs)] adjacent to the cell nucleus (top left). Scale bars: 200 μm (**A** and **B**); 2 μm (**C** and **D**); 500 nm (**E** and **F**).

virus replication occurs predominantly within the mesenchymal or supporting stromal cells of the reproductive tract. The presence of the virus in the ovary and uterus in the macaque model of EVD has not been reported previously. However, detection of EBOV in the thecal mesenchymal cells surrounding ovarian follicles and IHC positivity associated with inflammation in necrosis in the uterus have been demonstrated in the guinea pig model of EVD.[20] The effect of EBOV infection of thecal cells on the denouement of secondary and tertiary ovarian follicles in macaques is unknown. The effect of EBOV infection of smooth muscle cells in the uterus is also unknown; however, in pregnant women with EVD, an average maternal death rate of 82% and a fetal death rate of 100% have been reported.[21] Ebola virus is able to infect the fetus.[22] In the placenta, fetal-origin syncytiotrophoblasts can be infected with the Sudan and Bundibugyo viruses.[21] The only known congenitally infected infant to survive EBOV infection was treated on 2, 5, 8, and 11 days of life with the monoclonal antibody cocktail, ZMapp, and received an i.v. transfusion of leukocytes from an EBOV survivor on day 11 of life; beginning on day 19 of life, this infant was treated for 12 days with the nucleotide analog prodrug, GS-5734.[23] Ebola virus is shed in breast milk, and epidemiologically linked transmission from an asymptomatic mother to a 9-month-old child through breast milk has been reported.[24] The presence or absence of EBOV persistence in women is understudied, as are the effects of EVD in pregnant women and their offspring.

In men, temporal evidence and sequence analysis of EBOV samples from the 2014 to 2016 West Africa outbreak demonstrated sexual transmission of EBOV to women from 6 months to >1 year after recovery from EVD.[7,8] A recent report from Fischer et al[25] described the longest interval, 965 days, between onset of EVD and the detection of EBOV RNA in semen. The prevalence of detection was significantly higher in older men (median age, 41.8 years), and detection was intermittent. On the basis of our findings, we hypothesize that persistence is established in the interstitial tissues of the reproductive tract and tissue macrophages are the route by which EBOV is transmitted from stromal replication sites in the seminal vesicle, epididymis, prostate gland, and testis into the seminal fluid. Because this study used tissue collected adjunctively at necropsy from ongoing studies, the collection and examination of seminal fluid was not possible, and no fresh tissue was collected for RNA sequencing.

Infection of monocytes and macrophages facilitates systemic spread of the EBOV but neutrophils and B, T, and natural killer cells, critical for both the innate and humoral immune response, are not infected.[26] Although we have demonstrated active EBOV infection of endothelial cells in reproductive tissues, endothelial cells exhibit a high degree of heterogeneity, and endothelial cell infection within one tissue type likely does not translate to permissivity of viral infection in another.[27] Although the macaque model of human EVD is thought to have a high fidelity, it is unknown if the distribution of the virus is similar in the reproductive tract of affected humans or why sexual transmission in convalescent human populations is rare. Martines et al[28] reported positivity for EBOV antigens by IHC in testicular endothelium and focally within the seminiferous tubular cells in one man. Zeng et al[29] recently reported detection of EBOV viral RNA in the interstitial tissues of the testis and epididymis, in the same pattern we describe herein, at day 43 after EBOV exposure in 1 of 76 nonhuman primate EVD survivors after experimental countermeasure treatment. These findings support the need for an established definition of EBOV persistence in the macaque model of EVD and to determine whether the presence of viral RNA in the semen equates to the presence of infectious EBOV. The findings from Fischer et al[25] and Zeng et al[29] also highlight the need to evaluate multiple seminal samples from a diverse study cohort for the presence of EBOV to determine whether age affects the incidence of EBOV persistence. Proposed EVD countermeasures need to be evaluated for efficacy and to determine whether they facilitate the development of EBOV persistence.

Several RNA viruses that establish persistence in infected hosts may provide valuable insight into EBOV persistence.[30] Equine arteritis virus establishes persistent infection in stallions after recovery from clinical disease. The stromal cells of the ampulla, infiltrating tissue macrophages, T cells, and B cells in stallions are persistently infected, in the presence of neutralizing antibody.[31] Persistence of equine arteritis virus is associated with polymorphisms in the stallion's *CXCL16* gene. Virus shedding in seminal fluid continues for an average of 165 days after infection in the absence of viremia and clinical evidence of reproductive dysfunction. Although a genetic link associated with viral persistence of EBOV in humans has yet to be established, such a link might explain the rarity of seminal transmission of EBOV.

Survival from EVD has been associated with an early and robust $CD8^+$ T-cell response.[32] The presence of EBOV in the stroma or interstitial tissues of the reproductive tract may be one of several mechanisms by which the virus evades early antiviral T-cell responses in immune-privileged sites to facilitate the establishment of persistence. Up-regulation of the ligand for the immune inhibitory receptor, programmed cell death 1 (CD274) pathway, has been shown in immune-privileged sites, such as the seminiferous tubules of the testes in mice.[33] This pathway is thought to be key in modulating immune function through inhibition of programmed cell death 1−positive $CD8^+$ T lymphocytes.[33,34] Resident tissue macrophages in the interstitium of the testis exhibit an M2, or immunosuppressive, phenotype, which may facilitate the development of viral persistence.[35] The virus likely evades the immune system through several mechanisms, including the production of two proteins, VP24 and VP35, that inhibit the production of interferon α in antigen-presenting cells, thus attenuating T-cell receptor activation and T-cell expansion.[36,37] During infection, the virus down-regulates GP while concurrently releasing a soluble GP that binds circulating neutralizing antibodies directed against EBOV GP on cell surfaces.[38] *In vitro*, EBOV establishes noncytolytic persistent infections through the production of defective interfering particles.[39,40] A nonhuman primate EBOV survival model would be required to study the host immunomodulatory mechanisms that facilitate the development of EBOV persistence in immune-privileged sites and to determine how this highly pathogenic virus attenuates itself to persist in host cells.

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